ABSTRACT

Title of thesis: EVALUATING THE BIOSORPTIVE PROPERTIES OF ANACOSTIA RIVER BIOMASS TO FACILITATE HEAVY METAL REMOVAL FROM CONTAMINATED WATER

Team CYCLE, Gemstone Honors Program, 2021

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Arsenic, cadmium, and chromium are among the major industrial heavy metal pollutants that can cause adverse effects on human and environmental health. Conventional remediation treatments tend to be financially and environmentally disadvantageous. Algal biosorption is an alternative that utilizes the functional groups on algae's surface to remove metals from solution. We tested the remediating capabilities of algae in both a laboratory and prototype setting. We observed how arsenic, cadmium, and chromium were sorbed by the algae at select time intervals. We found that 100% of chromium and arsenic and 35% of cadmium were removed after 24 hours, with peak rates occurring for all three metals at two hours. Results from the prototype show promise, but shortcomings suggest this technology is better suited for use in pretreatment, not for immediate discharge. More research is needed to improve the system's practicality in real world application.

EVALUATING THE BIOSORPTIVE PROPERTIES OF ANACOSTIA RIVER BIOMASS TO FACILITATE HEAVY METAL REMOVAL FROM CONTAMINATED WATER

by

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2021

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List of Abbreviations

WHO - World Health Organization
EPA - Environmental Protection Agency
Cr - Chromium
As - Arsenic
Cd - Cadmium
Pb - Lead
$CaCl_2$ - Calcium chloride
HCl - Hydrochloric acid
HNO_3 - Nitric acid
ICP - Inductively coupled plasma
ICP-OES - Inductively coupled plasma-optical emission spectrometry
RSD - Relative standard deviations
Aq - Aqueous
NaOH - Sodium hydroxide
NaCN - Sodium cyanide
mM - Millimolar
DI water - Deionized water
PVC - Polyvinyl chloride (pipes)
mL - Milliliter
PPM - Parts per million
mg - Milligram
L - Liter

1 – Introduction

Heavy metal contamination has steadily increased around the world in the last half century[9]. In a study conducted across five continents from 1972 to 2017, researchers found that the average concentrations of heavy metals in the world's waterways have generally been higher in the last 30 years than they were in the previous 20, and the gross number of heavy metals exceeding World Health Organization (WHO) and United States Environmental Protection Agency (EPA) threshold limits for surface water has risen[9]. In 2015, nearly 21 million Americans relied on community water systems that violated health based environmental standards[10]. Heavy metal pollution poses a life-threatening and economically challenging obstacle to human and environmental health[11]. Even at concentrations as low as 0.005 mg/L, heavy metals can be toxic and induce organ damage; many are classified as human carcinogens[11]. Additionally, they are a danger to the health and wellness of the plant and animal species that survive off the polluted waterways[12].

Most environmental contamination is a result of human activities such as mining, industrial manufacturing, and agriculture[11]. One example of severe contamination is the Cuyahoga River, which runs through Cleveland, Ohio. As Cleveland grew to be a major manufacturing hub of the Midwest, its river became severely polluted. In 1969, the river famously caught fire, causing financial and environmental damage[13]. Love Canal, a neighborhood in upstate New York, is another example. This development was partially built on top of a heavily polluted chemical dumping site. Health problems arose and

persisted in the area, including cancer and birth defects[14]. These environmental disasters caused by industrial manufacturing led to the environmental movements of the 1970s and ‘80s, which led to the establishment of the EPA, the Clean Water Act, and the Superfund program[15]. However, thorough regulatory oversight like this often may not exist in low income areas, both domestically and abroad, where economic development is often prioritized over environmental concerns[16]. That means these communities are more threatened by contaminated water sources, and thus more vulnerable to the adverse effects of heavy metal pollution.

Leather tanning, the process by which leather is cured, generates significant amounts of heavy metal waste. Over 60% of the world’s leather is produced in developing countries[17]. Animal hides are exposed to a number of heavy metals, primarily chromium (Cr), in order to stabilize, soften, and protect the leather[10]. Each ton of leather produces 0.12 kg of Cr waste, along with other common heavy metal contaminants, including arsenic (As) and cadmium (Cd)[12,18]. For developed countries like the United States, the acceptable concentrations of these metals are minute; the EPA standards for Cr, As, and Cd, are 0.1 mg/L, 0.01 mg/L, and 0.005 mg/L, respectively[19]. Less developed countries tend to have less enforcement of environmental regulations, such as Bangladesh, where tannery sludge is haphazardly disposed of in landfills or dumped openly, resulting in soil, surface water, and groundwater contamination[20]. This pattern is repeated over and over in a number of industries and countries, disproportionately harming the people and environments of these countries. The impacts of heavy metal pollution are far reaching; as seen in the stakeholder analysis in Section 7.2, there are

many communities and organizations who are touched by or involved in the contamination of water with heavy metals. The extent of this issue necessitates an easy and effective method for heavy metal removal.

Ever since heavy metals were identified as toxic contaminants during the 20th century manufacturing boom, researchers have continued to advance methods for their detection and removal. The key factors for determining the success of any heavy metal removal method is its effectiveness and its environmental and economic favorability[5]. Conventional treatments that meet these standards include electrochemical cells, ion exchange, and chemical precipitation[21]. However, success in remediating heavy metals has a tradeoff: treatments are expensive, energy intensive, unsustainable, and may generate other harmful chemical contaminants such as hydrogen sulfide gas[22,23].

The three common methods to treat contaminated water are chemical precipitation, electrochemical cells, and ion exchange[24]. Most commonly used is chemical precipitation, which may require one or several chemical additives depending on the conditions of the wastewater[24]. The chemical precipitation process introduces a chemical reaction which produces a solid precipitate. A coagulant binds itself to the impurities in the solution, trapping unwanted ions. Unfortunately, chemical precipitation is a high maintenance process; it needs constant manual oversight and input to perform efficiently[25]. The electrochemical process relies on generating an electrical current within the solution[26]. This process releases charged ions into the solution, destabilising the unwanted particles. Despite seeing a resurgence in recent research, the electrochemical process usually results in secondary pollution and low-grade results[27]. The electrodes also

corrode over time, necessitating expensive replacements. The ion exchange process involves a chemical reaction where a smaller, less toxic, ion replaces the heavy metal in water[3]. Due to the additives introduced into the water, the pH is raised while the particle density stays the same, which may lead to other issues with the water safety[25].

Biosorption is an emerging treatment method that shows promise in regards to the problems previously identified. It uses biological agents such as bacteria or algae in remediating heavy metals from water. Biosorption's use of low-cost, renewable biomass makes it a favorable alternative to conventional methods[5,28]. Heavy metal contaminants are often found in a liquid or semi-liquid state, contributing to their pervasiveness in public waterways, and making their removal a necessity. Because biosorption occurs in aqueous solutions, it follows that this may be an effective treatment method for removing heavy metals from water and making them easier to dispose of. The abundance of algae in aquatic ecosystems means that it is both easily accessible and sustainable in diverse environments. In many places, climate change and agricultural runoff have caused excess growth of algae. These algae blooms can emit foul-smelling odors and may lead to eutrophication which causes dead zones where no plant or animal life can grow; in severe cases this leads to species extinction[29]. Using this excess algae presents an exciting opportunity to recycle excess biomass. This project reused local excess algae from nutrient capture systems to remove heavy metals from contaminated water.

The idea of recycling algae fits into a broader purpose of making our project simply one step in the larger water treatment cycle. As can be seen in Figure 1, this project fits in between the harvesting of biomass and the removal of heavy metals.

We sought not to introduce anything new to the environment that is being worked in. Instead, we used resources already at our disposal, which reduced the potential for said resources to become waste or cause harm. Recognizing that this project is one step in a larger process reinforces the desired sustainable outcomes and gives the research more applicability to varied ecosystems, where resources and problems will undoubtedly differ.

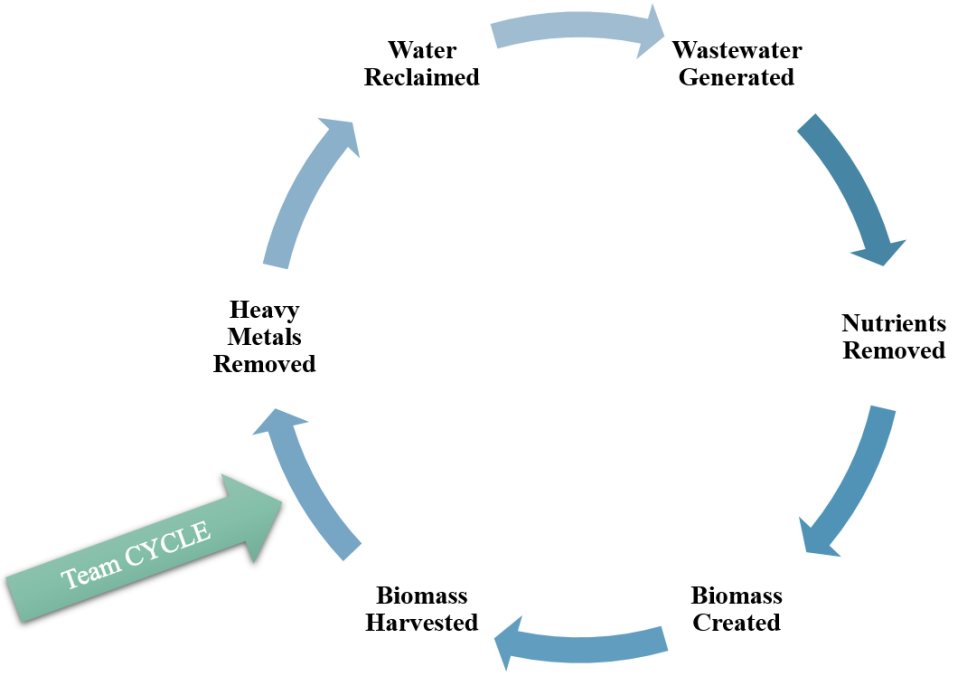


Figure 1. A visualization of Team CYCLE's research in regards to the larger water cycle.

The project's first aim was to evaluate the biosorptive properties of algae grown and harvested from the Anacostia River in Maryland. Experiments were conducted to determine the preferred conditions, contact time and chemical pretreatment, at which the algal biomass facilitates optimal heavy metal removal. These findings elucidated the biosorptive potential of the local algae. The second aim was to develop a prototype system incorporating native algae sourced from other research projects that effectively

removes heavy metal contaminants in a timely and cost effective manner. We hope that these findings serve to inform further research and system designs that may be employed to protect at-risk communities from heavy metal related illnesses or by industries, such as leather tanneries, who are looking for a cheaper option to clean up their manufacturing byproducts.

These two aims of our project can be captured in the following questions:

(1) What are the optimal contact times and chemical pretreatment for heavy metal removal with local algae?

(2) How can a system proof-of-concept be designed that removes heavy metals from contaminated water using native algae?

Based on existing literature, we hypothesized that adsorption capacity will occur after 4 hours, and that a pretreatment process using calcium chloride will improve the adsorptive capacity of the algae. Meanwhile, we explored different prototype designs to expand upon previous research and our own lab testing in order to determine the practicality of algal-based heavy metal removal systems.

2 – Literature Review

2.1 Algae Background

Algae was provided from the lab of Dr. Peter May, assistant research professor in the University of Maryland's Department of Environmental Science and Technology. It was harvested from a nutrient capture system, known as an algal turf scrubber, in the freshwater Anacostia River. The community is made up of several species of algae and diatom, since it grows naturally in situ with no environmental control. No microscopic study was conducted, but prior studies predict that the community used in this project consisted primarily of the diatom genus *Melosira* and algal genera *Cladophora* and *Spirogyra*[30]. The algae provided was sun dried for 1-2 days, which is beneficial for algae biosorption as dead biomass does not need any nutrients to sustain and can be exposed to toxic environments, such as heavy metal contaminated waters[53].

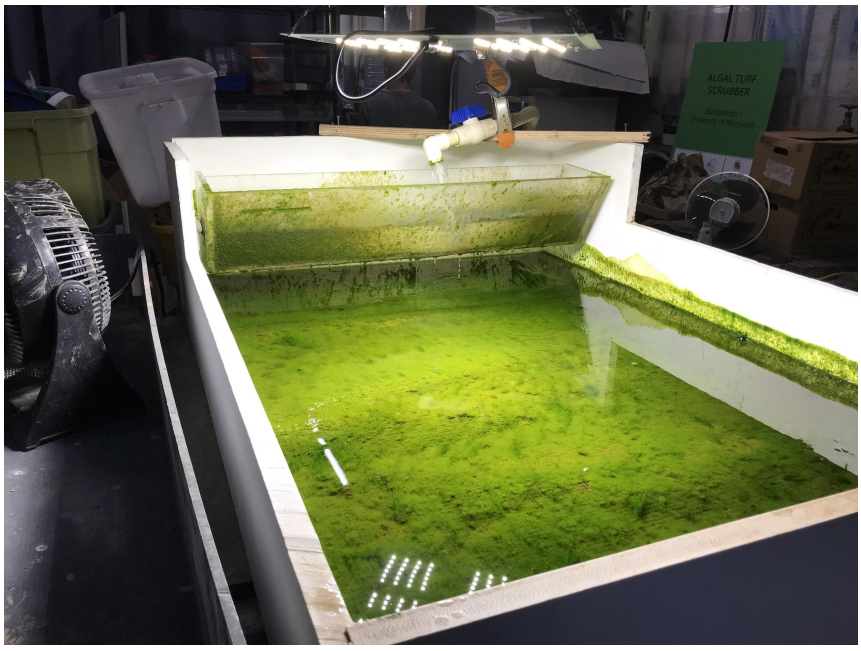


Figure 2. Algal turf scrubber in the lab of Dr. Peter May, UMD. Picture, Ben Baitman, December 6, 2018.

2.2 Algae as a Biosorbent

Biosorption research often focuses on algae, diatoms, and cyanobacteria as sorbents for heavy metal removal. These organisms can capture heavy metals through absorption, where heavy metals are contained inside the cell, and adsorption, where heavy metals adhere to the surface of the cell[31,32].

2.3 Functional Groups and Biosorption

In algae, functional groups are found along the cell wall on sugar complexes known as polysaccharide accessories. The most common polysaccharide accessories in algae are alginates and fucoidans. Alginates and fucoidans are embedded with carboxylic acids, hydroxyls, and sulfates (Figure 3). These functional groups are highly reactive and readily form complexes with the charged metal ions, even after the algae becomes inert[33]. There are two main methods by which metal binding mechanisms can occur: ion exchange and chemisorption[34].

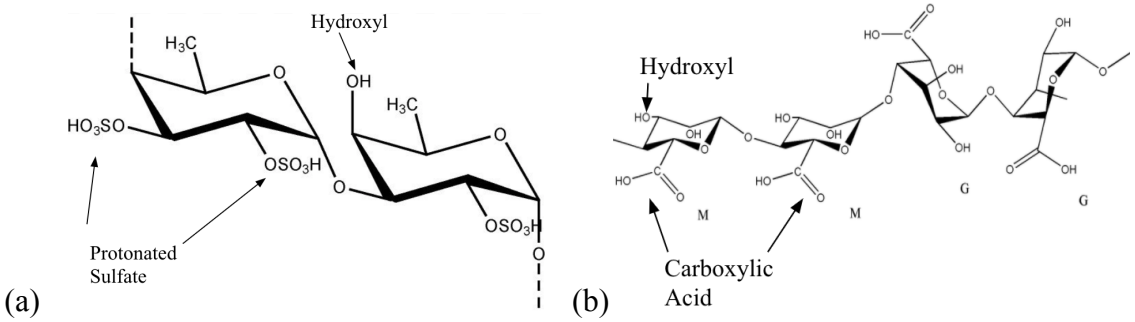


Figure 3. Fucoidans (a) and Alginates (b) include reactive functional groups such as carboxylic acids (COOH) and sulfates (HSO_4^-)[1,2].

2.3.1 Ion Exchange

Ion exchange occurs when a substrate binds an ion from the surrounding medium, generally a solution. Ion exchange substrates are stable at high temperatures and function across a wide variety of pH values. Ion exchange substrates contain a wide variety of functional groups, from highly acidic groups like sulphonates to strongly basic groups like ammonium[35]. These reaction mechanisms are reversible, and can be used to adsorb both cations and anions. Ion exchange is driven by electric potential, such that electroneutrality is met[36]. It is important to note that the ion exchange is equivalent. Ion exchange can be modelled well by both pseudo first and second order reactions, and occurs very quickly, due to the fact that no electron bonds are broken during the process[37].

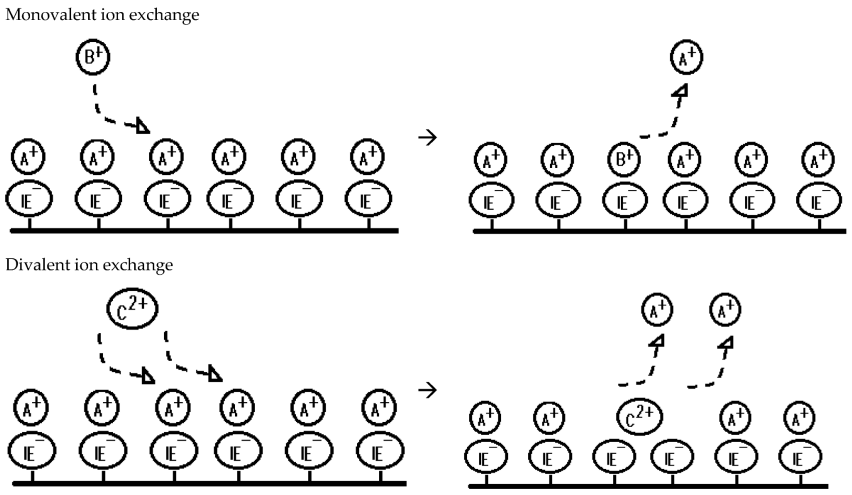


Figure 4. Example of the ion exchange process[3].

2.3.2 Chemisorption

Chemisorption, unlike ion exchange, forms a chemical bond between the substrate and the heavy metal ion. In chemisorption, the substrate's surface is lined with binding sites, where heavy metal ions attach via covalent or ionic bonds. Chemisorption substrates can adsorb at nearly any temperature, but the temperature range of the ion may be limited. This sort of adsorption can be dissociative, and as such can often be irreversible. Due to the kinetic breaking and formation of new bonds, the speed of chemisorption is extremely variable[38].

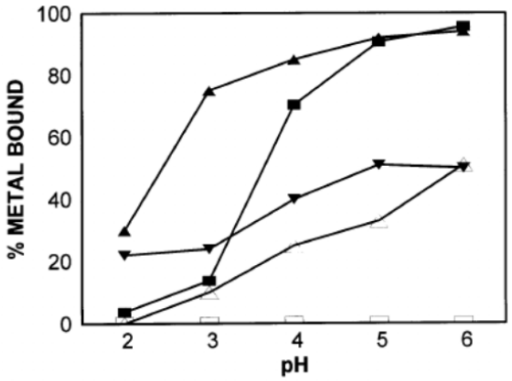
2.4 Parameters Impacting Functional Groups

In both ion exchange or chemisorption, functional groups only become active bonding sites under certain conditions of their chemical environment. The availability of polysaccharide functional groups is dependent upon pH, biomass concentration, surface area, contact time, and pretreatment.

2.4.1 pH

pH is a major parameter when examining biosorptive effectiveness and capacity. Both the binding affinity of algal functional groups and heavy metals are affected by ambient pH conditions. Depending on the target metal ion, functional groups become optimally active at different pH levels. Less reactive ions require more acidic conditions to form complexes with the polysaccharide accessories while some ions require more basic pH levels that activate functional groups such as hydroxyls[23]. A majority of heavy metals tend to precipitate out at pH values higher than 9, making it unlikely for the metal to adsorb onto the algal surface[39]. Additionally, the optimal pH level for maximum

percent removal of heavy metal ions has been observed to lie around 5 to 6 in the figure shown below (Figure 5). The pH level is maintained at 5 for the lab experiment, within the optimal range of 5 to 6, in order to facilitate a higher percentage of heavy metal removal.



EFFECT OF pH ON THE BINDING OF CADMIUM(II) ■, CHROMIUM (III) ▼, CHROMIUM (VI) □, LEAD (II) ▲, AND ZINC (II) △ BY AFRICAN ALFALFA SHOOTS. BIOMASS (5 mg/ml) WAS SHAKEN FOR ONE HOUR AT THE APPROPRIATE pH WITH 0.1 mM OF EACH OF METAL ION, INDEPENDENTLY.

Figure 5. The percent removal of metal by African alfalfa shoots is observed to peak at the slightly acidic pH level of 5 to 6 (adapted from [4]).

2.4.2 Biomass Concentration

Biomass concentration (or biomass dosage), generally measured in grams per liter, describes a solution's ratio of biosorbing algae to a unit volume of solution containing heavy metals. Higher biomass concentration dramatically increases ion removal capacity which levels off around 10 g/L for both Cr and lead (Pb) (Figure 6). Therefore, a biomass concentration of 10 g/L was used in the lab experiments.

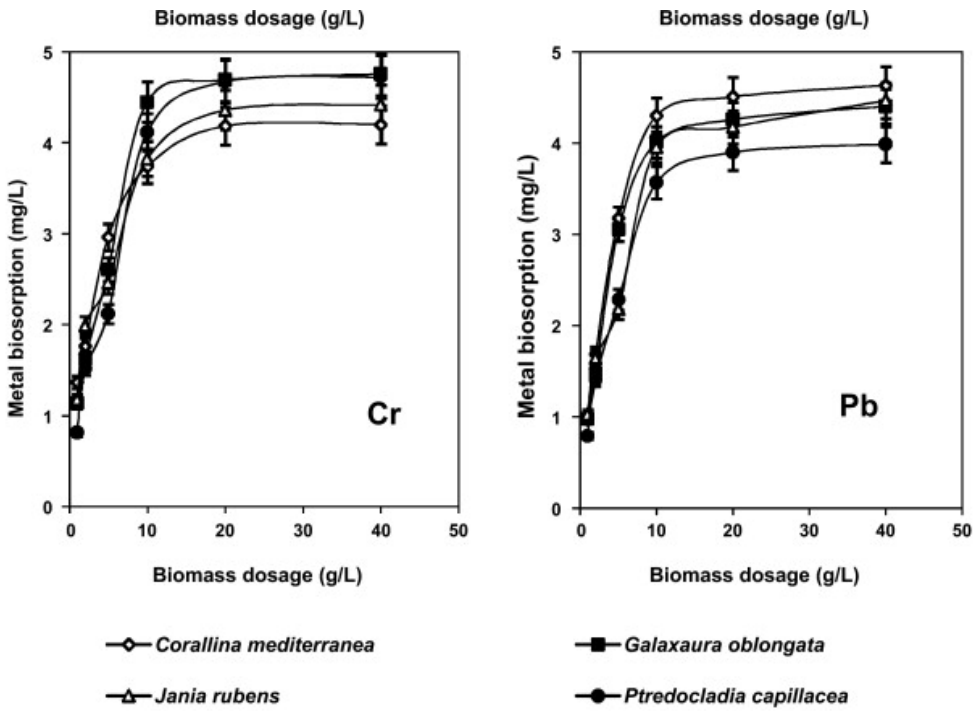


Figure 6. Ion removal capacity increases with biomass dosage, then levels off at a peak concentration[5].

2.4.3 Surface Area

Surface area is the total exposed area on the algae that is actively sorbing heavy metal ions. Algae with a higher surface area has more active functional groups on its cell wall that are available for complexation with metal ions, becoming a more effective biosorptive material (Figure 7)[6]. The figure below shows the relationship between the surface area to mass ratio of the algae compared to the concentration of the metals removed. With the same amount of algae, smaller particles have a higher surface area than clumps of algae. Because surface area has an inversely proportional relationship to particle size, the algae was finely grounded using a mortar and pestle to ensure maximum adsorption.

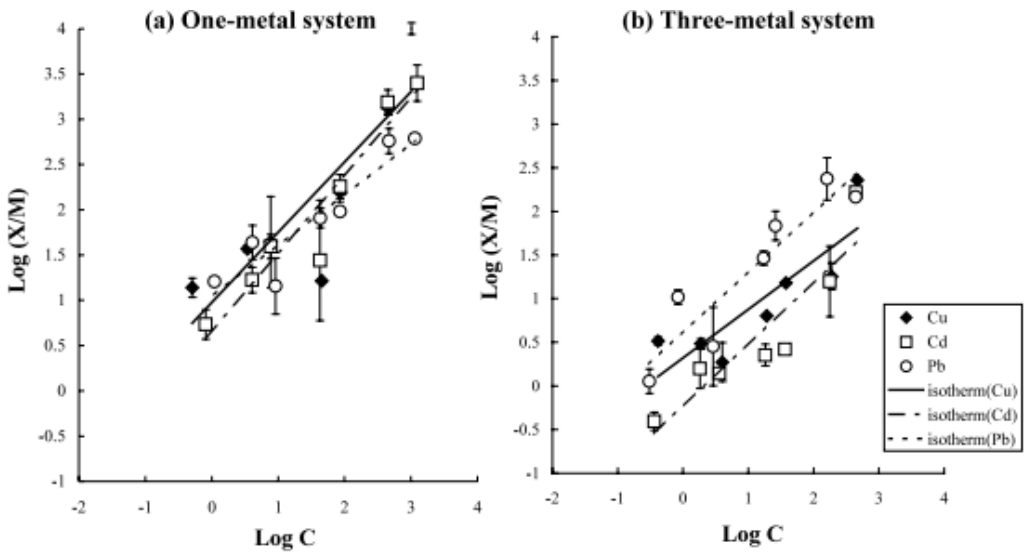


Figure 7. The amount of heavy metals adsorbed increases with a larger amount of biomass present[6].

2.4.4 Contact Time

The amount of time a sorbent is submerged in an ion concentration is known as contact time. The functional groups on an algal surface take time to bind with the surrounding metal ions in a given solution. This can be modelled via chemical kinetics. The rate constant *k* is used to predict the rate of conversion from reactants to products in a chemical reaction. In algal biosorption systems, the interaction between algae and metal ions can be modeled as a chemical equation:

Algae + Metal Ion → Algae-Metal Complex

In algal biosorption, *k* therefore predicts the rate at which algae captures metal ions. *k* is related to both *A*, the concentration of the heavy metals, A_o, the initial concentration of the heavy metals, and *t*, time. This reaction is usually assumed to be first order, as most algal functional groups interact with a single metal ion. However, *k* values

corresponding to both first and second order reactions will be calculated and compared for accuracy. The derivation of *k* will be based on the following rate-law equations[40]:

$$\text{First order: } \ln[A]t = -kt + \ln[A]_0$$

$$\text{Second order: } \frac{1}{[A]} = \frac{1}{[A]_0} + kt$$

As with biomass concentration, the amount of metal ions adsorbed by an algal sorbent is initially proportional to the length of time it is in contact with the solution (Figure 8). While there are no binding sites occupied, the only limiting factor for adsorption is the amount of time it takes for a metal ion to bind to the surface, which is constant. However, as more and more metal ions bind onto the surface of the algae, another limiting factor presents itself: competition for binding sites. Therefore, as a majority of active functional groups become occupied, the rate at which metal binds to the sorbent decreases, and an overall plateau in sorption capacity is shown (Figure 8). In the lab methodology, the point at which this plateau is reached will be determined in order to maximize the amount of heavy metals removed in the shortest amount of time.

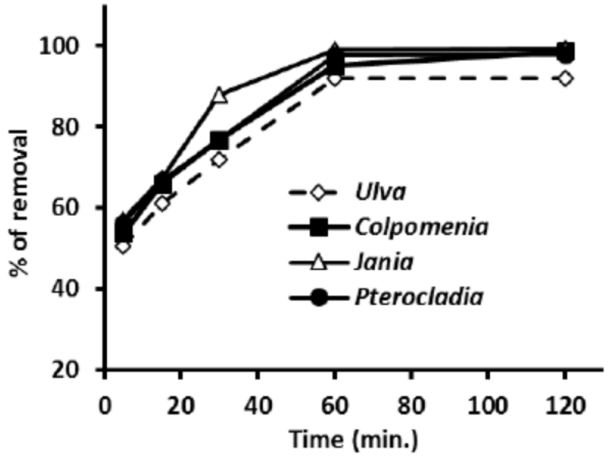


Figure 8. Relationship between contact time and percent removal of Cd^{+2} (adapted from [7]).

2.4.5 Pretreatment

Functional groups are often occupied by preexisting complexes with light metal ions, like calcium, aluminum, and magnesium. These complexes can prevent the binding of target heavy metal ions. Pretreatment is one method of disrupting existing complexes and promoting heavy metal capture. A pretreatment is conducted on algae by soaking it in a chemical solution for a period of time in order to open up more binding sites that may be occupied. The most popular agent for pretreatment is calcium chloride ($CaCl_2$). Not only is $CaCl_2$ economical, but it also significantly increases the biosorptive capability of a wide variety of algae. For example, a pretreatment in 0.2 M $CaCl_2$ for 24 hours increased the biosorptive capacity of *Spirulina maxima* by 84–92%[41]. Mineral acids like HCl and HNO_3 have also been used for pretreatment, being highly effective for a smaller variety of algae. Due to the effectiveness of $CaCl_2$, a pretreatment of the local algae with $CaCl_2$ was conducted in the lab to determine whether local algae will experience a significant increase in biosorptive capacity compared to the standard treatment.

Pretreatment works by having an ion bind onto the alginates. This plays an important role in ion exchange. When using $CaCl_2$, the calcium is strong enough to bind onto the surface of the algae, but weak enough that it will be replaced by a heavier metal. HCl works in a similar way; however a proton replaces the light metal ions on the surface of the metal. Pretreatment prepares these functional groups to facilitate ion exchange more efficiently.

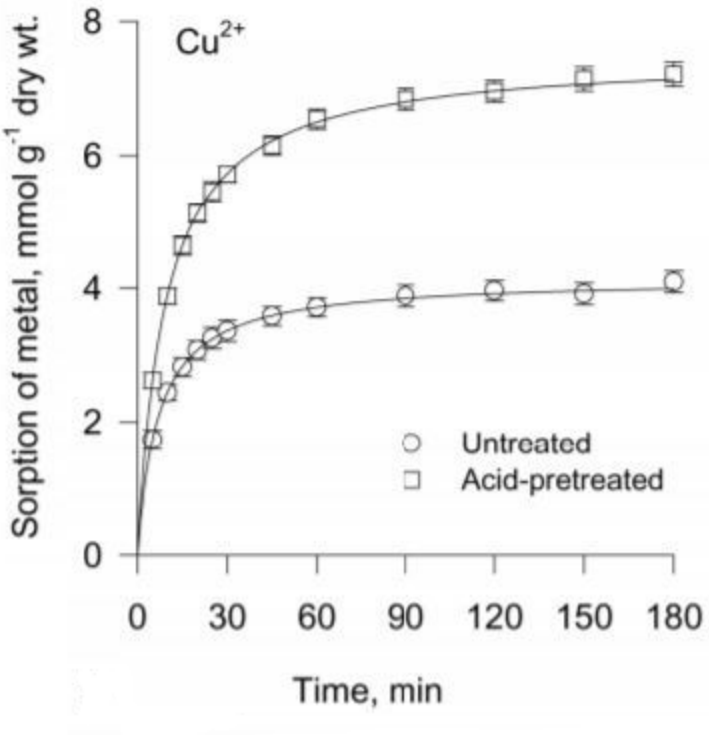


Figure 9. Amount of metal sorbed by algae with and without $CaCl_2$ pretreatment (adapted from [8]).

3 – Methodology

The methodology is broken down into two distinct parts: the laboratory experimentation and the prototype development. The lab methodology was a series of experiments used to identify the ideal parameters for heavy metal removal and provide answers to our first aim. The prototype development, informed by the lab methodology, was used to answer Aim 2. All samples were filtered and analyzed with a Shimadzu ICPE-9000 Multitype Inductively Coupled Plasma (ICP).

3.1 Lab Methodology

3.1.1 Algae Composition

Freshwater algae was acquired from Dr. Peter May, who grew it in an algal turf scrubber using water from the Anacostia River. The dominant genera of algae were *Cladophora* and *Spirogyra*. The diatom genus *Melosira* was also highly prevalent[30].

3.1.2 Algae Preparation

The algae provided was dried in the sun for 1-2 days (Figure 10). After drying the algae, the samples were ground in a ceramic mortar and pestle. The algae was kept in a glass container, covered with parafilm.

Figure 10. Algae after drying the sun for 1 to 2 days. Picture, Paul Seibert, February 26, 2021.

3.1.3 Solution Preparation

The heavy metal solution was prepared with Cr(aq) in 2% HNO_3, Cd(aq) in 2% HNO_3, As(aq) in 2% HNO_3, HNO_3 (98%), and NaOH (98%). All chemicals were purchased from Inorganic Ventures in Christiansburg, VA. The solution included As, Cd, and Cr at a concentration of 0.1 mM (millimolar) each and was adjusted to pH 5 using nitric acid (HNO_3) and sodium hydroxide (NaOH) according to optimal adsorption conditions mentioned in section 2.4.1[5].

3.1.4 Parameter Testing

3.1.4.1 Biosorption Procedure

The experiments were carried out in 250mL Erlenmeyer flasks filled with 100mL of 0.1 mM heavy metal solution[42]. 1g of the prepared biomass was then added to the flask to prepare a final solution with a biomass concentration of 10 g/L[42]. The flask was sealed

with parafilm and shaken at 80 rpm in room temperature[5]. The flasks were removed at different elapsed times: 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, and 24 hours. The solution was then filtered through Whatman No.1 2.5 micron filter paper and its heavy metal concentrations were analyzed through inductively coupled plasma - optical emission spectrometry (ICP-OES). The biomass' percent biosorption capacity for heavy metal ion removal was calculated using the following formula[5]:

$$\text{Biosorption (\%)} = \frac{(C_i - C_f)}{C_i} \times 100$$

C_i – initial concentration, C_f – final concentration

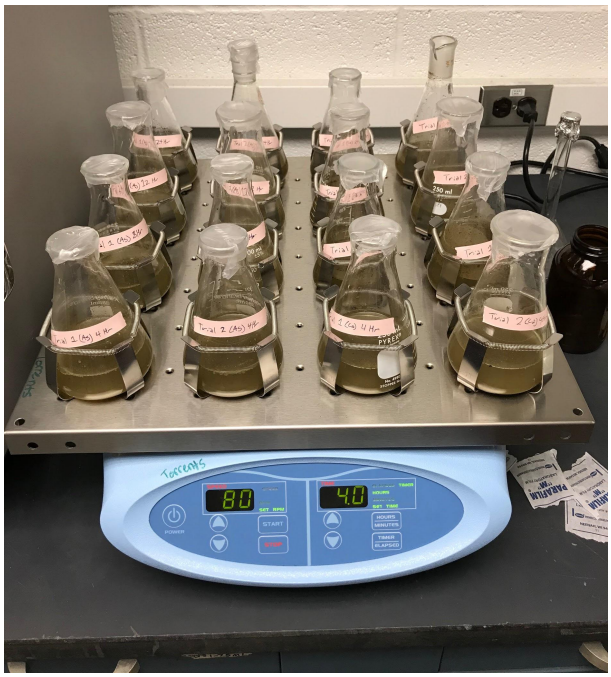


Figure 11. Flasks with 10 g/L of biomass prepared for contact time experiments. Picture, Ben Baitman, October 12, 2019.

3.1.4.2 Pretreatment

A different set of experiments were done to determine the effect of pretreatment on biosorption. Prior to the addition of biomass, algae was shaken for 24 hours in 0.2 M $CaCl_2$ solution at 80 rpm. Afterwards, the algae was washed with deionized water and baked at 80ºC for 24 hours. The algae was then finely ground in a mortar and pestle and kept in a glass container covered in parafilm. Adsorption of heavy metals using pretreated algae followed the biosorption procedure described in Section 3.1.4.1.

3.1.5 Detection Limits

A method detection limit protocol was run to determine the lower quantitation limits of the metals we tested. DI water was spiked with 5 and 10 ppb of each metal. 7 replicates of each concentration were run. The standard deviation was taken and multiplied by 2.5 as per standard protocol to obtain the lower quantitation limits[43]. The limits were as follows: As, 0.46 ppm; Cd, 0.12 ppm; and Cr, 0.09 ppm.

Additionally, lab trials were run with inert media to rule out any unexpected factors impacting the metal concentration in samples. The protocol followed the general biosorption procedure outlined in section 3.1.4.1 but the metal solution was run with lab-grade sand instead of algae. Control samples were run through the same method with DI water. No significant deviation from the initial metal concentrations was detected.

Standard checks were also run to ensure the quality of the data was consistent. Standard solutions with predetermined metal concentrations and blanks (DI water) were run every 10-15 samples during ICP analysis.

3.2 Prototype Development Methodology

The second aim of this project was to construct a prototype system to facilitate the removal of heavy metals from industrial stormwater runoff using locally-sourced algae. Most of the literature suggesting the use of algal biomass for biosorption ends at lab experimentation. Thus, our focus was to evaluate options for expanding towards prototypes that could be tested for use in industrial settings, such as leather tanneries.

Decisions regarding the system designs were made based on the preliminary results of the lab experiments. Because the movement of water through the system would sacrifice contact time compared to water in flasks, the ratio of algae mass to heavy metal solution was tripled. The increase in algae mass proportional to the amount of solution allowed for a greater surface area of algae to interact with the heavy metals. It was predicted that this would increase the adsorption rate allowing us to quantify the heavy metal removal in the smaller timescale of the system. These modifications were made to the system in the hopes of gaining comparable results to the lab experiments

3.2.1 Construction of Prototype Systems

Three prototype designs were considered in this project: a sloped baffle system, vertical baffle system, and vertical cartridge systems. For all of the prototypes, design parameters were based on flow rate, the volume of water capable of being treated, integration of algal biomass, and materials of the build.

Design 1 - Sloped Baffle System

Figure 12. Construction of sloped baffle system. Picture, Bryan Croce, November 5, 2019.

The sloped baffle system utilized four aluminum baffles lined with stainless steel mesh to hold algae. On initial testing, there was significant issue with the flow of water. The bottoms of the baffles were not water-tight and efforts to seal the gaps proved ineffective. Additionally, water was only flowing on the bottom end of the baffle, resulting in little of the algae being part of the flow path. Furthermore, the construction of the stainless steel baffles was considerably more difficult than other pre-built materials, such as PVC, resulting in slow modification time. After these trials, the system was no longer tested or altered further.

Design 2 - Vertical Baffle System

Figure 13. Vertical Baffle System. Picture, Paul Seibert, February 5, 2021.

The vertical baffle system utilized a similar layout to the sloped design but used PVC as the baffle base. Sleeves made out of cheesecloth were made to be filled with algae and rest on the PVC track. The gaps between the PVC and the wood base were filled using an all-purpose water sealant. Initial tests were encouraging, but the integration of the algae led to leaks and other safety concerns. The surface tension of the cheesecloth bags caused the water to bounce off the top of the bags due to insufficient back-pressure in the water stream. Similar to the sloped baffle system, any water that

made it past the cheesecloth did not flow through the biomass, resulting in poor efficiency. These downsides caused the system to be dropped from consideration.

Design 3 - Vertical Cartridge System

Figure 14. Testing the Vertical Cartridge System. The top cartridge is loaded with algae, while the bottom cartridge is left empty to collect water for easy sampling. Picture, Joseph Weller, February 24, 2021.

This design included three PVC filter casings in series that operate under bulk flow of fixed volume. Each casing was open at the top and able to be connected to the next cartridge using PVC couplings. At the bottom of each cartridge, a sheet of stainless steel mesh (N = 120) was laid with a lining of grade 90 cheesecloth on top to ensure ponding occurred within each cartridge. The stainless steel mesh and cheesecloth were purchased through Amazon and sold by TIMESETL and SCENG, respectively (see section 7.3 for links). The ponding induced a stable flow rate through the cartridge due to

a fixed level of hydraulic head, standardizing the contact time. Within each cartridge, a fixed mass of algae (284 grams) was placed on top of the cheese cloth lining to ensure that ponding would occur. During loading, the algae was compacted twice, at 200 and 284 grams, using a clean section of PVC to gently compress the algae down.

Figure 15. Loading the algal biomass to cartridge B. Picture, Bryan Croce, February 26, 2021.

3.2.2 Sampling of Prototype System

In addition to the three main cartridges, a control cartridge was prepared under the same conditions to be run with DI water. This was analyzed to determine if any metals were contributed by the system or algae. To test heavy metal removal, 4.72 L of 0.1 mM metal solution of Ar, Cd, and Cr were run through the system utilizing a 9.4 L plastic water jug with a variable spigot (see top of Figure 14). The metal solution was run through cartridge 1 at a rate 3.2 L/min for 20 seconds to allow ponding to occur, then 0.86 L/min for the remainder of the sampling period.

Figure 16. Samples with system cartridges. Picture, Bryan Croce, February 26, 2021.

Samples 10 mL in volume were taken every 30 seconds at the bottom of the empty cartridge as shown in Figure 17, starting 30 seconds after beginning the flow of water. Eight samples were collected in 30 second intervals afterwards. The final sample was taken at the end of the run. The cartridge was removed and replaced with the second cartridge, and the entire volume of heavy metal water was then reloaded into the plastic water jug. The sampling process was repeated for each cartridge. The control cartridge followed the same scheme, but was limited to six samples. To prepare for ICP analysis, the samples were syringe filtered using a Whatman GD/X filter with a pore size of 1 micron (Cytiva, Marlborough, MA).

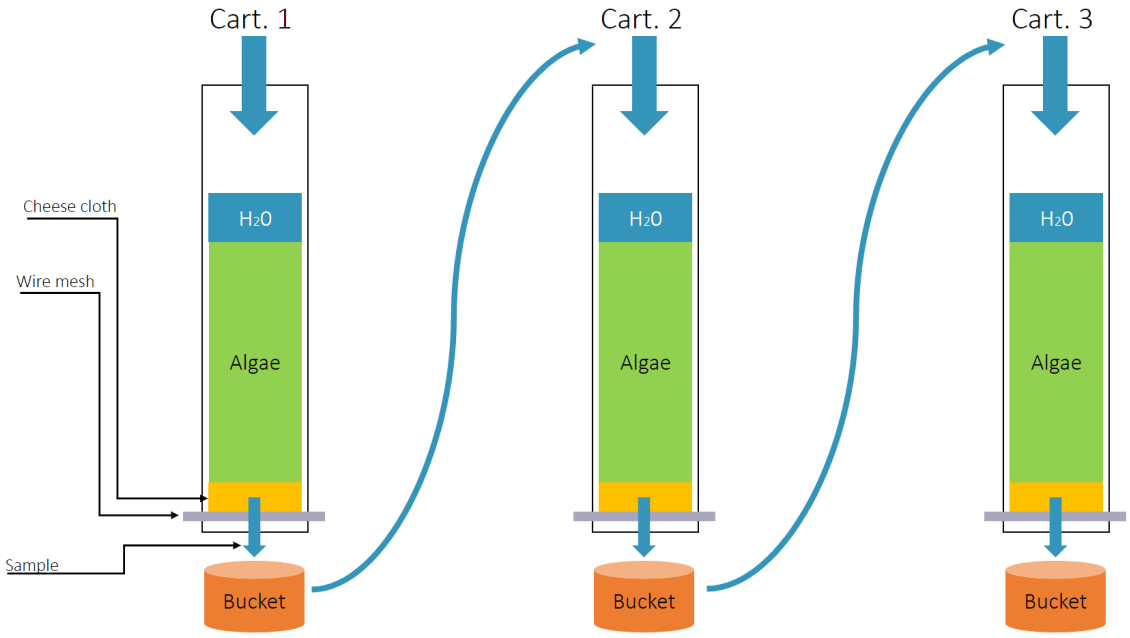


Figure 17. System Testing Method.

4 – Results

All samples were filtered and analyzed with a Shimadzu ICPE-9000 Multitype Inductively Coupled Plasma (ICP). Samples that were found to be outliers by the Grubbs test or had relative standard deviations (RSD) greater than 2 were omitted from further analysis.

4.1 Lab

Initial tests were completed in the lab to generate a baseline understanding of the performance of the algae collected. Figures 18 and 19 show the adsorption of heavy metals in terms of concentration and percent removal, respectively. Results indicated that arsenic adsorbed the fastest onto the biosorbent, followed by chromium. Percent removal for arsenic and chromium reached 100% after 24 hours, while cadmium only reached 35% removal.

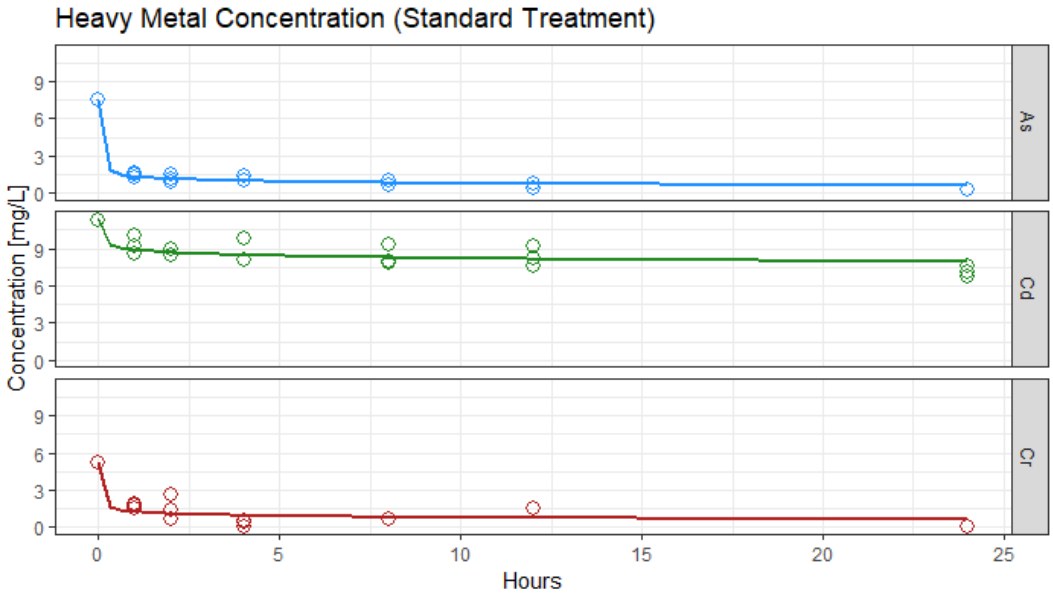


Figure 18. Metal concentrations by trial number over a 24 hour period (Standard treatment).

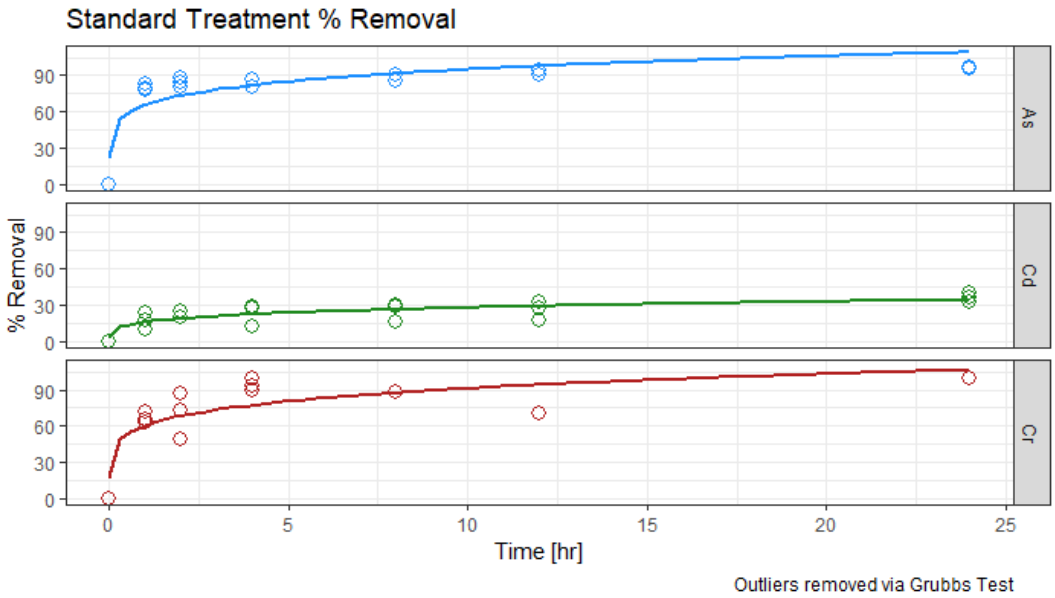


Figure 19. Percent Metal Removal (Standard Treatment).

For the purposes of this study, the optimal rate of adsorption will be defined as the point at which algal adsorption rate is minimized, or in other words, the point at which the algae has reached its maximum adsorption capacity. The optimal rate of adsorption can be determined by plotting the derivative of Figure 19, and examining when the slope approaches zero. Because the slope of the graph will only approach zero and never reach it, we deem any rate less than or equal to 0.25 mg/L hr^{-1} as "approaching zero," which is visible on the graphs as a sharp change in rate. As shown in Figure 20**,** this rate is reached at the two hour mark for arsenic, cadmium, and chromium in the standard treatment.

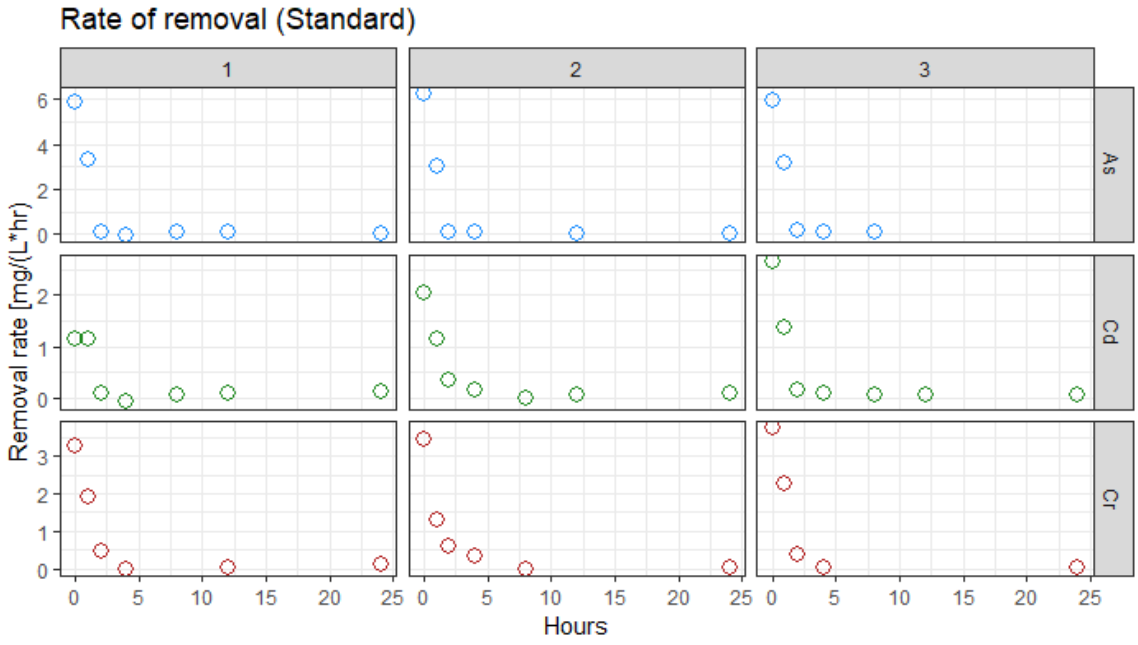


Figure 20. Rate of removal (Standard).

Each of the equations in Figure 21 represent a model by which the adsorption capacity and mechanism can be analyzed. The adsorption mechanism was determined by examining the kinetics of the reaction. The order of the reaction was analyzed by comparing the initial concentration to the corresponding differential in the rate of reactions. Based on Figure 21, the pseudo first order reaction yielded a large R^2 (between 0.89 and 0.95) values for arsenic and cadmium, indicating relatively good agreement between the data and the predictive model. The p-values for first order regression of As and Cd were both much less than 0.05 at a 95% confidence level, indicating that the first order model is statistically significant. The R^2 value for the chromium pseudo first order reaction was significantly smaller, 0.55. The p-value for 1st order regression of Cr was 0.28 at a 95% confidence level, indicating that the model is not statistically significant.

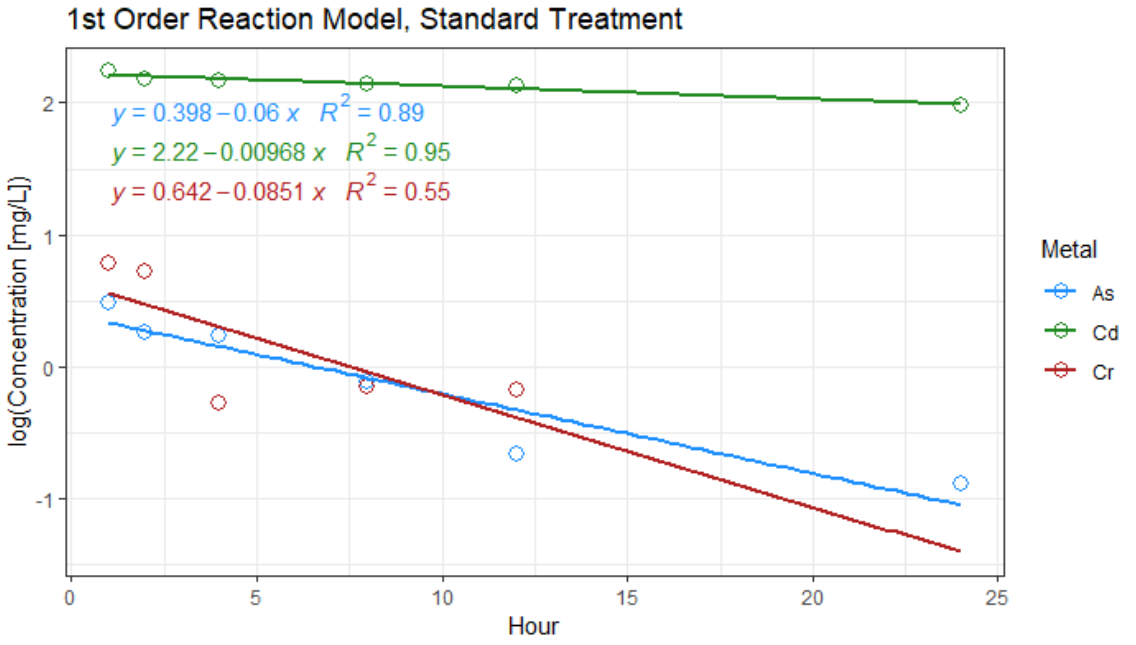


Figure 21. Standard treatment modeled as a 1st order reaction.

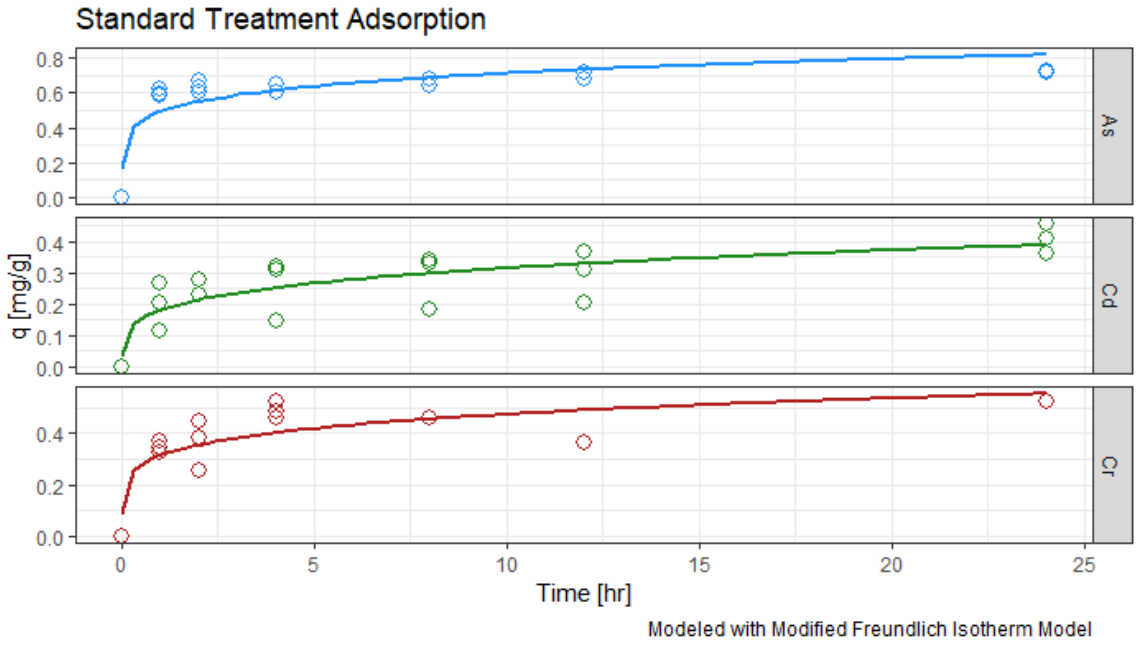


Figure 22. Standard Treatment Adsorption.

The standard treatment algae adsorption was determined using batch reactor equations derived and used by Desta[44]. The results are shown in Figure 22. This graph

was transformed into a log-log graph to derive Freundlich Isotherm coefficients following the same procedure as Tang et al[45]. This resulted in the coefficients in Figure 23.

k_f	1/n	Metal
0.599	0.056	Arsenic
0.188	0.208	Cadmium
0.350	0.120	Chromium

Figure 23. Freundlich Isotherm coefficients (Standard).

These coefficients are useful in the design of future adsorption columns through the Freundlich Isotherm Model $q = kf * C^{(1/n)}$. This model is commonly used in environmental engineering for designing adsorption treatment systems and deriving design parameters, as done in a study with Tang et al. [45]. The Freundlich model assumes that time is constant and concentration (C) of the adsorbent is varied. The use of this model in this batch reactor study is justified because time was varied and C was constant.

Pretreatment was also analyzed as an option to maximize adsorption of heavy metals over time. Shown below in Figures 24 and 25 are the adsorption for cadmium, chromium, and arsenic with pretreated algae in terms of concentration and percent removal, respectively. Based on Figure 25, the pretreated algae removes about 90% of the arsenic, 30% of the cadmium, and 80% of the chromium in solution after 24 hours.

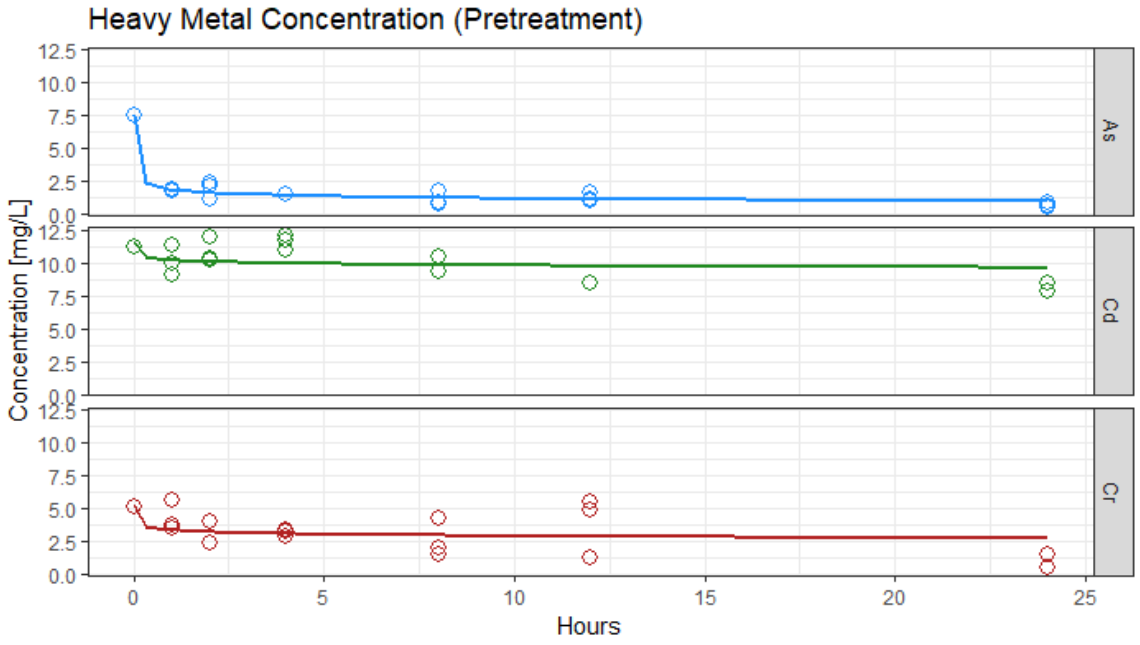


Figure 24. Metal Concentration over a 24 hour period (Pretreatment).

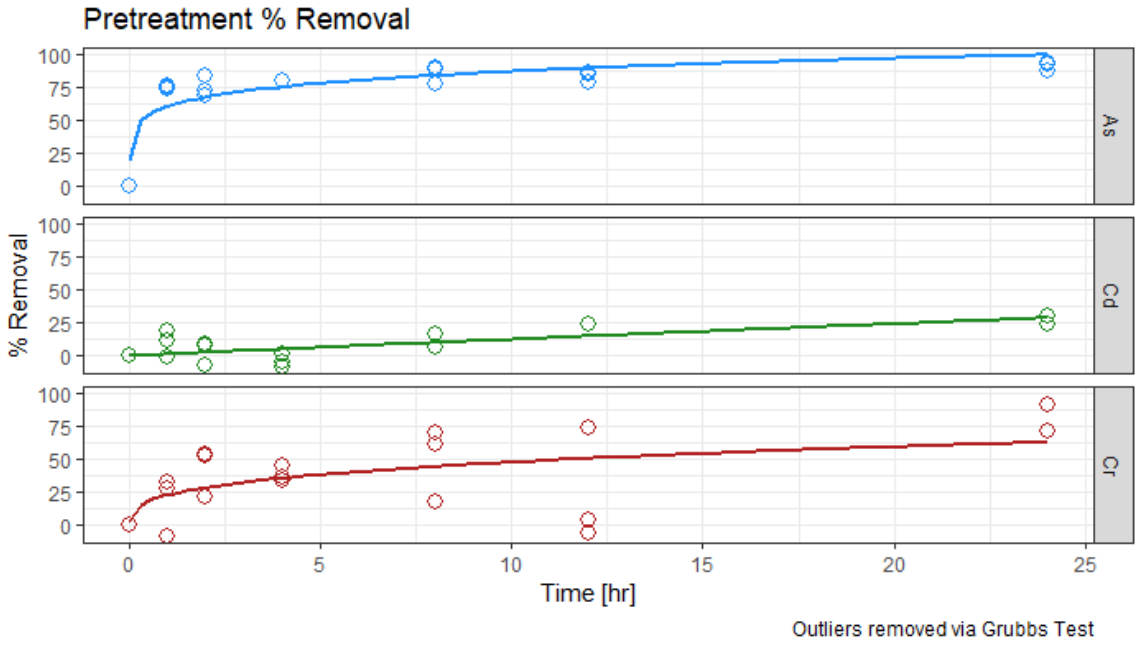


Figure 25. Percent Metal Removal (Pretreatment).

The order of these reactions were analyzed by comparing the initial concentration to the corresponding differential in the rate of reactions. Based on Figure 26, shown below, the reaction orders for arsenic, cadmium, and chromium have similar trends to the reaction orders for those metals in pretreated algae. However, the R^2 for each metal is lower than their counterparts in the standard treatment. P-test analysis revealed that the first order model is statistically significant for all metals at 95% confidence; p-value < 0.05.

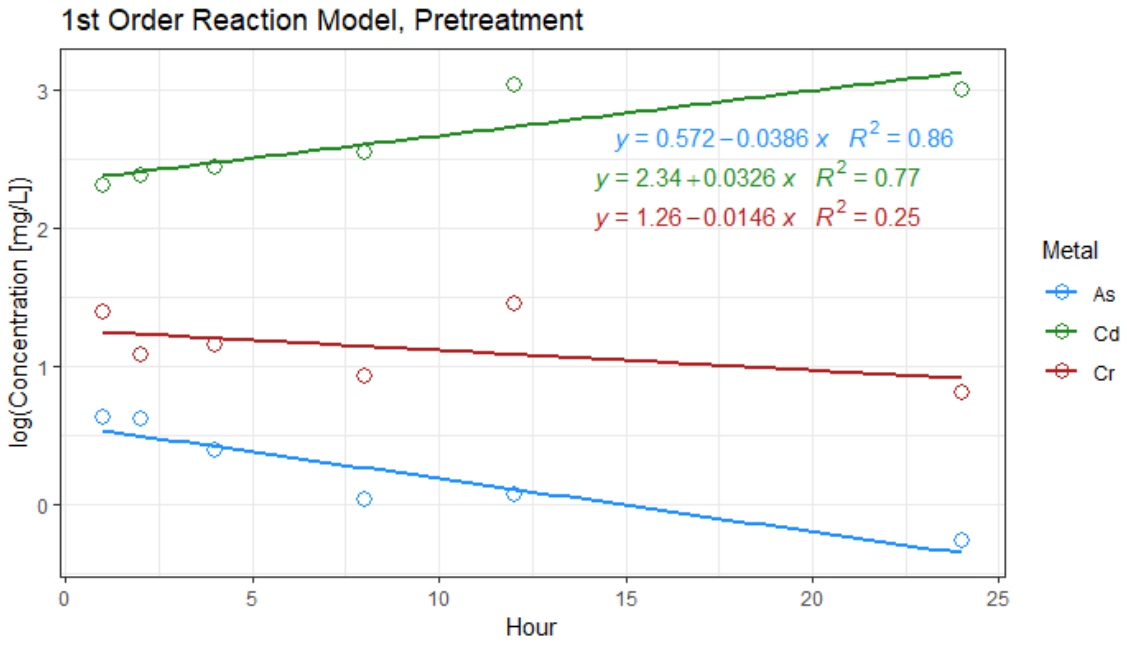


Figure 26. Pretreatment modeled as a 1st order reaction.

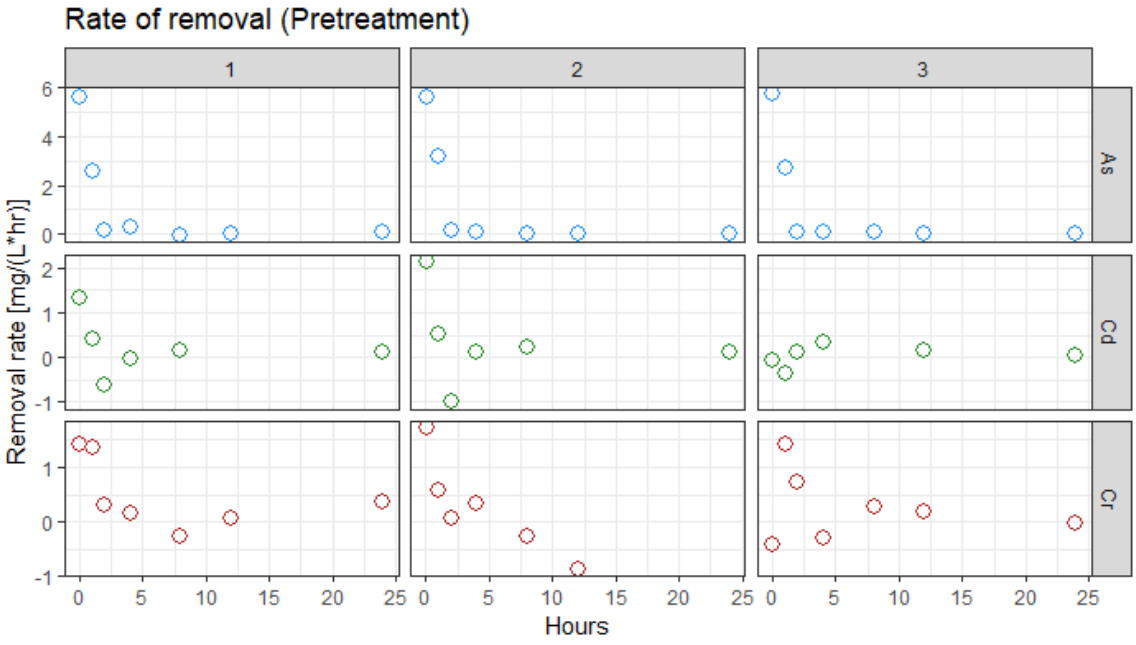


Figure 27. Rate of removal (Pretreatment).

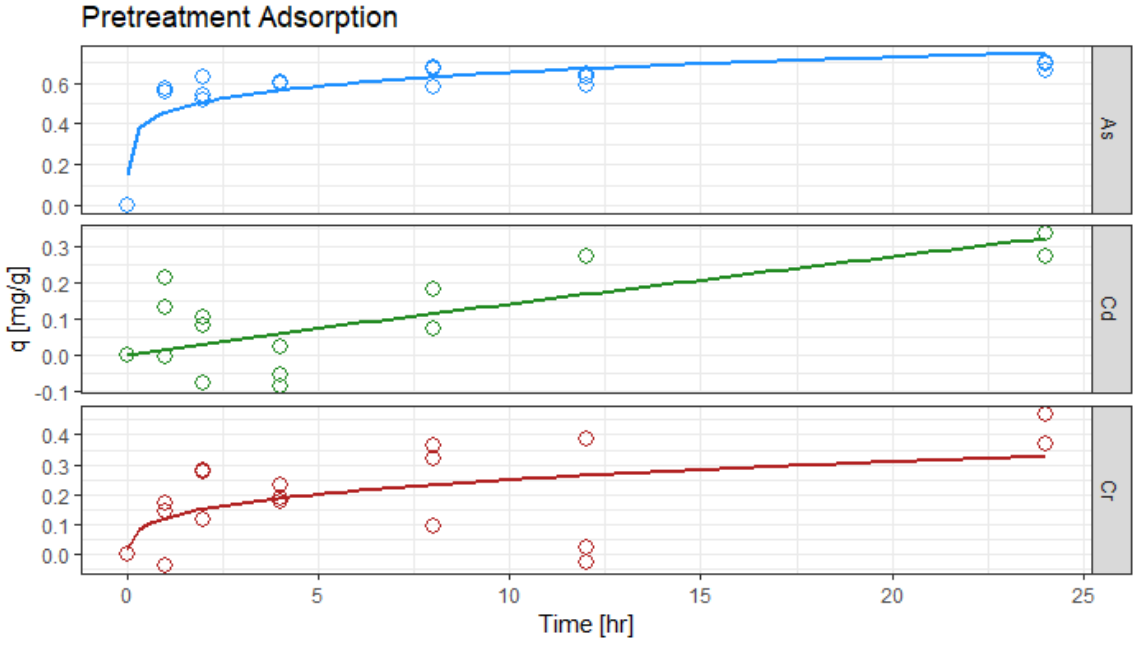


Figure 28. Pretreatment Adsorption.

The adsorption of metal ions onto the pretreatment algae was analyzed in the same procedure as the standard algae, which resulted in Figure 28. Freundlich analysis resulted in coefficients for the pretreatment algae, shown in Figure 29.

k_f	1/n	Metal
0.550	0.062	Arsenic
0.086	0.307	Cadmium
0.162	0.124	Chromium

Figure 29. Freundlich analysis coefficients (Pretreatment).

4.2 Prototype

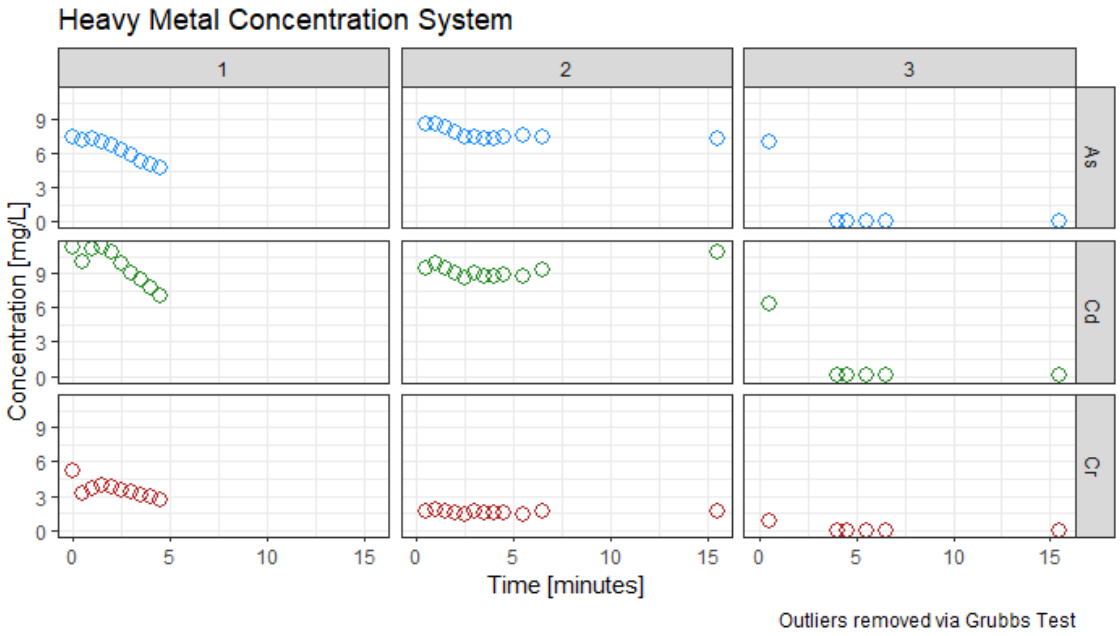


Figure 30. Metal concentration (System) by cartridge.

The amount of time required to finish the volume of water for each cartridge was not uniform, with significant variance occurring in cartridges 1 and 2. The run times

were: 15.5, 28, 5.75, and 6 minutes for cartridges 1, 2, 3, and control, respectively. For consistency, samples up to 16 minutes were utilized for graphing and analysis.

Figure 30 shows the concentration of metals over time by cartridge number, where water passes through cartridges in sequential order (1-3). In the first cartridge there is a sharp decline in metal concentration for all three metals. Arsenic decreased by about 2.5 mg/L in the first 5 minutes while passing through the first cartridge. The same occurs with Cadmium, which decreased by about 3.5 mg/L in the first 5 minutes while passing through the first cartridge. Chromium decreased by around 2 mg/L in the first 5 minutes when passed through the first cartridge. After passing through all cartridges, nearly all of the arsenic, cadmium, and chromium had been removed. In Figure 31, the percent removal of metals is shown between the three cartridges over time. Based on these results, it can be shown that the prototype was able to absorb around 100% of the original concentration of all three metals.

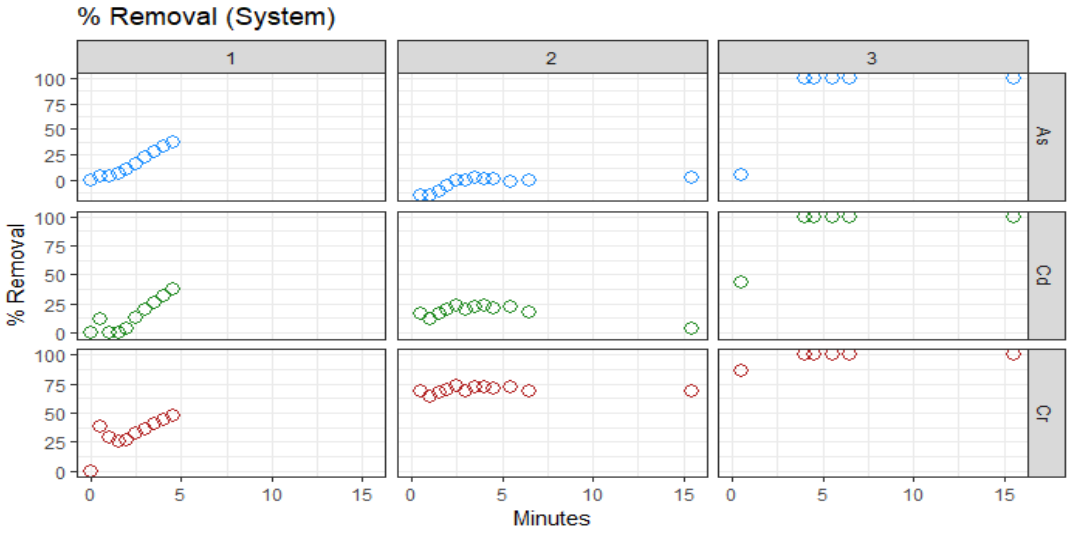


Figure 31. Percent removal of metals by cartridge.

5 – Discussion

5.1 Lab Trial Insights

In this study, optimal contact time was defined as the point at which the rate of adsorption drops below 0.25 ppm hr^{-1}. The optimal contact time for most efficient adsorption varied slightly by metal, but peaked at or before 2 hours. This suggests that in a prototype using a biomass concentration of 10 g/L of Anacostia river algae should be replaced with new algae every 2 hours, where every metal starts being removed at less than 25 mg/L hr^{-1} for an efficient rate of adsorption. Other studies reached an optimal adsorption interval in under an hour using much lower biomass concentrations; for example, a study using *Chlorella vulgaris* had an optimal contact time of 20 minutes using a biomass concentration of 1g/L[46]. Conversely, as biomass concentration increases, a longer contact time would be necessary to reach adsorption capacity. It is worthwhile to note that for these metals, the concentration fell below the quantification limit (as described in Section 3.1.5) sometime between the 12 and 24 hour intervals, so it cannot be said with any certainty what the actual percent removal may have been. The detection limit for As and Cd was greater than the maximum concentration allowed by the EPA, and was about equal for Cr[47]. Therefore, we suggest that an algal biosorption system is insufficient for discharge into waterways and should be used as a pretreatment of contaminated water before it is processed by a wastewater treatment facility, which do not typically have heavy metal removal methods in place.

Based on adsorption rate and percent removal, the untreated algae generally followed or exceeded adsorption trends found in the literature[6,7,42]. As, Cr, and Cd achieved adsorptions of 0.633, 0.361, and 0.245 mg/g algae respectively by the optimal time at 2 hours. Nearly 100% of As and Cr were removed in the 24 hour interval, reaching a final adsorption capacity of 0.709 mg/g of As and 0.520 mg/g of Cr. Cd was adsorbed at a lower rate up to 35% removal (equivalent to 0.408mg/g) in 24 hours, which is consistent with other studies that compared adsorption rates of multiple metals[48]. Cd in particular is known to be highly soluble and has a high affinity for dissolved solids, which may further explain its lower rate of removal as compared to As and Cr[49,50].

As shown in Figure 21, the pseudo first order reaction model acted as a good fit for both arsenic and cadmium, but not chromium. It is reasonable to say that the mechanism of adsorption is ion exchange due to the good agreement with the first order reaction model, as cited in the literature[37]. It would be reasonable to conclude that conditions that would increase the efficiency of an ion exchange reaction would likewise increase the efficiency of heavy metal removal in this prototype. This would include an increase in temperature of the water, an increase of concentration of heavy metals, and a slow enough flow rate such that ion exchange has enough time to occur.

In contrast, the pretreatment algae did not perform as well compared to the standard treatment. By the 24-hour mark As achieved an adsorption of 0.682mg/g algae, Cd 0.316 mg/g, and Cr 0.420 mg/g. All of the metal concentrations fluctuated significantly throughout the experiment, especially Cd. The fluctuation of Cd concentrations may be due to the pretreatment releasing pre-existing Cd from the algae

back into the solution. Although there was generally a downward trend in concentration, it is too tenuous a relationship to consider a success. There are a few possible reasons for why the results were so variable. It is possible that rather than enhancing the adsorption capacity of the algae, $CaCl_2$ may have interfered with the binding sites in some way. Additionally, the rinsing step after the treatment with $CaCl_2$ could have sloughed off binding sites from the algae, leading to poorer adsorption. Other studies found that pretreated marine algae was highly successful in adsorbing lead[51,52] or that cyanobacteria benefitted from pretreatment[41], but it is possible that $CaCl_2$ is antagonistic to other metals and/or diatoms and freshwater algae, which were the major components of our algae. Additionally, poor accuracy of ICP readings is possible. Though the samples were vacuum filtered before analysis, there is a potential for dissolved solids to interfere with the equipment and oversaturate the plasma, causing inaccurate readings. The high RSD values and frequent nebulizer cloggings that coincided with the outliers and unexpected values certainly indicate some inaccuracy with the readings. Therefore, we do not recommend that pretreatment with $CaCl_2$ be used on algae similar to the ones used in this study. However, pretreatment should still be tested with other types of algae as many literatures with pretreatment have been successful in increasing the adsorption capacity of the algae. Other types of pretreatment should also be studied.

Dissolved solids containing heavy metals are not only a concern for laboratory equipment; this may also be an unanticipated concern of algal treatment of wastewater. As dissolved solids decompose, these metals may be rereleased into the environment. These dissolved solids were present in both the samples with pretreatment and standard

treatment. Therefore, it is recommended that where sufficient filtration is not possible, the algae should not be finely ground as it may contribute to higher levels of dissolved solids. However, dissolved solids may be preferable over toxic heavy metals for wastewater treatment facilities as they may be easier to treat and the dissolved solids also concentrate the heavy metal ions. This also further affirms that algal biosorption is a tool suited to prepare wastewater for a treatment facility, but not for immediate discharge into the environment.

5.2 Pilot System Insights

Similar to the lab results, there were unfortunately difficulties with the instrumentation due to dissolved solids. Because of clogging and other interference, the samples had to be diluted 1:10 and run separately in small groups, and the calibration curves were not consistent. As a result, the data appears disconnected and is likely not representative of the actual metal concentrations in the samples.

The sampling method of the prototype was designed to determine the heavy metal concentration of the water directly as it exited each cartridge. Doing so would provide insights on the performance of the cartridge and indicate where the rate of adsorption would begin to decline. Given this design, it was expected to see steady or slightly increasing concentration data points as the adsorptive capabilities of the biomass were being used. However, the results do not follow that expectation but offer more uncertainties. In cartridge 1, the decrease in concentration of all three metals would suggest that the extended run time (15.5 minutes) resulted in more adsorption opportunity. But, cartridge 2 showed a constant removal rate (see Figure 31) despite

almost double the run time (28 minutes). The extended run time is likely due to the reuse of the cheesecloth filters used in each cartridge, which caused the cartridge to be clogged with algae. These unexpected results make it difficult to accurately judge the effectiveness of the system and would require additional testing to validate the data.

The system design has clear advantages. A primary benefit of the cartridge system is its modularity; since each filter cartridge is uniform, construction of numerous casings can be performed easily and maintenance for each one is the same. Additionally, casings can be arranged in series or parallel to increase capacity while meeting the physical needs of the treatment site. Furthermore, filter cartridges are not new in design, so institutional knowledge regarding design, operation, and maintenance of this system has analogs to existing filtration systems.

However, some limitations must be addressed. A major shortcoming of this system is the need to replace the algae once adsorptive capabilities are low. Typical filter media, such as slow and rapid sand filters in wastewater treatment methods, utilize backwashing (pumping water through the system in the opposite direction) to clean and extend the usability of the media for many years. However, this system is biologically immobilizing the heavy metal, resulting in the need to physically replace the biomass in order to continue filtering wastewater. Due to the labor required, it is most practical to replace biomass on the order of hours or days; the resources required to continuously remove and replace the algae may make such a system impractical. However, the design may be suitable for specific waste streams that can support the resource demand of labor and algal biomass.

Another shortcoming of the prototype was the high variability in flow rate between the cartridges. Some cartridges took upwards of 15 minutes to drain, while others drained in around six minutes. This variability was due to the cheesecloth in two of the cartridges being clogged, constricting the flow path. In the left image of Figure 32, there is very little room for water to flow, which caused clogging in the second cartridge. In contrast, the right image of Figure 32 shows that water flowed easily through areas of the third cartridge, where algae had not clogged the system. A possible explanation of this is that the cheesecloth used in cartridges 1 and 2 had been run with algae prior to testing with heavy metals, while the cheesecloth in cartridges 3 and 4 was brand new at the time of loading. This suggests that the reuse of the cheesecloth between runs is correlated to clogging.

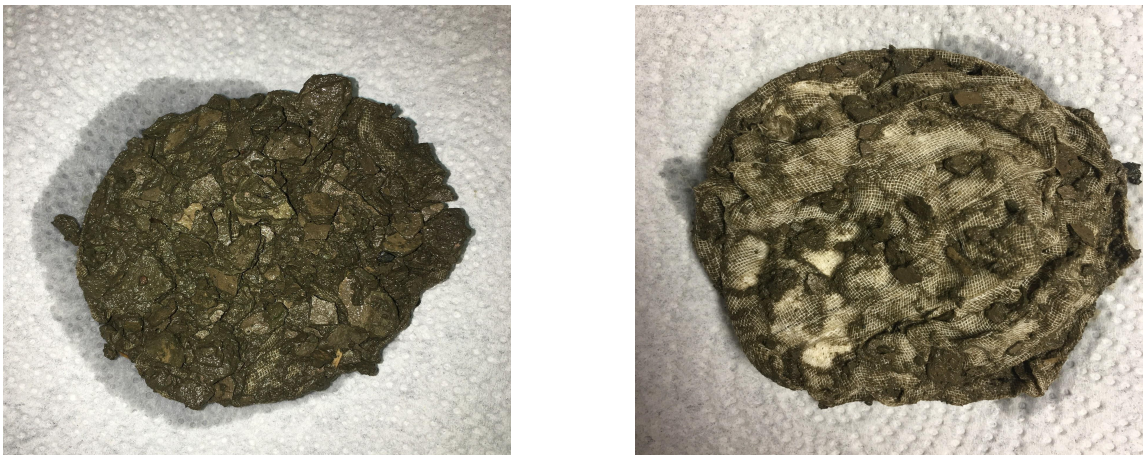


Figure 32. Image of the cheesecloth inside of cartridge 2 (left) and cartridge 3 (right). Cartridge 2 ran for 28 minutes, while cartridge 3 ran for just under 6 minutes. Picture, Paul Seibert, February 26, 2021.

In practice, significant variability could cause severe inefficiencies in the system or even cause backups to occur. Two potential ways to address this issue are to ensure

uniformity of algal particles using sieves, or to pressurize the system to force water through at a steady flow. In both cases, additional resources are required to add these modifications, increasing the associated costs and resource demand.

5.3 Limitations

Several factors significantly disrupted our project's timeline, which limited the data we could collect and prevented us from testing prototypes in the way we had intended. These factors included the COVID-19 pandemic, which limited the use of laboratory equipment and prevented most face-to-face collaboration. Additionally, just as our first round of samples was ready for analysis, the previous ICP machine on campus broke down, forcing us to mail our samples to a lab, which took up a greater share of our time and funding than we anticipated.

As a result of the truncated timeline, the lab results could not inform the prototype as much as planned, since we had not finished collecting data for the lab by the time the prototype was constructed. Therefore, the prototype data is somewhat disconnected from the lab data. Due to the different time intervals, biomass concentrations, and hydraulics, the ability to draw any major conclusions between the two aims is limited. In terms of data interpretation, several issues and malfunctions with the instrumentation made accurate and consistent results nearly impossible. We suspect that a high amount of dissolved solids interfered with the nebulizer and saturated the plasma. A handful of samples were lost due to leakage in the tubing. The method detection limit and the breadth of the calibration curve prevented more accurate assessment of samples with a low concentration. Additionally, the readings for the standard checks were below the

actual concentration in nearly every case, indicating that the data may have strayed further from the actual values as the runs went on. Finer filtration or dilution could have reduced clogging and oversaturation. A greater number of trials and samples may have helped to counter losses and outliers.

Outside of the limitations of our research, one of the most significant aspects of algal biosorption of heavy metals is the disposal of the algae after making capturing metals. Chemical precipitation is an option to reclaim metals, but its cost and environmental impact defeat the purpose of using algae in the first place. Having heavy metals adsorbed onto algal tissue is certainly preferable to having them dissolved in water, but this solution is only temporary and as the algae decomposes, it will release the metals back into the environment. In short, while algae can effectively remove heavy metals from water, it is not a sufficient long-term solution for reducing heavy metal pollution without additional processing. Fortunately, there are methods available to release the captured heavy metals, allowing the algae to be cycled back into the prototype for biosorption. For example, over 90% of gold was recovered from algae using a solution of either NaOH or NaCN[53].

5.4 Potential for Future Research

Further research should be conducted to determine whether a combination of diatoms and algae, such as the composition of the local algae used in this research, is more effective at heavy metal removal compared to only diatoms or only algae. Due to time constraints, the effect of biomass concentration on the performance of local algae was not determined in this study. Research into how biomass concentration, pH, and

surface area impact the biosorptive potential of local algae can be done to better inform the prototype design. Additional experiments can be conducted with the prototype where the used biomass is replaced by new biomass at the optimal contact time of 2 hours. This will inform the optimal number of cycles needed to reach threshold heavy metal removal.

Additional parameters can also be further expanded upon, such as the trade offs between particle size and the amount of dissolved solids. Smaller particle size results in a higher surface area, allowing more heavy metal ions to come into contact with functional groups on the algae. This will lead to a high adsorption capacity of the algae; however, a smaller particle size will also result in higher amounts of dissolved solids in the solution, thus warranting a better filtration system. Studies can be conducted to determine at what point the increase in adsorption capacity from smaller particles size is not worth the trade offs in increased amounts of total dissolved solids.

One of the intents of this project was to design, build, and assess the ability of multiple different prototypes to adsorb heavy metals. Unfortunately, two out of the three designed systems were never tested with heavy metal solution due to difficulties in flow control. Based on the research that was conducted, there appears to be potential to create new prototype designs and continue the prototyping process. It is recommended that future research looks into the applicability of the prototype in the actual industrial process, such as where the prototype would fit in the tannery wastewater treatment process.

6 – Conclusion

Even in minute concentrations, heavy metals can cause serious and long-lasting health and environmental problems. With heavy metal pollution being particularly prevalent in low-income and developing areas, and existing remediation techniques tending to be complex and expensive, a cheap and sustainable method for removing these contaminants from vital water ways must be developed. As a widely accessible medium, algal biomass presented itself as a possible low-cost and efficient solution, with sustainable applications that allow for the research outcomes to fit into existing water treatment cycles. The biosorptive capability of algae was tested in a controlled lab environment, where it was found to be an effective biosorbent. A series of prototypes that could take advantage of algae's biosorbing capabilities and be implemented in a real world setting were then developed. Results show heavy metal removal occurring in the third design, but significant modifications still need to be made in order to develop a practical system that can be used in a variety of settings. While the current results show promise in metal removal, more research must be aimed at building a prototype with less issues concerning absorbed solids and the uniform use of algae.

7 – Appendix

7.1 Stakeholder Analysis

Stakeholder	For, Against, Neutral (+, -, 0) Why?	Power (0 to 4, lowest to highest)	Cultural Values	Communication Strategy
Tannery Industry	+, Potential to save money, more sustainable 0/-, need to get algae, pretreat it, and dispose of algae waste product	4	$$$$, approval, efficiency, privacy, security	Send detailed report/thesis
EPA	0/+, depends on how much it really helps limit pollution	2.5	Environment, rule of law, standardization	Ask for permission/ regulations. Ask for local data
People living downstream of tanneries	+, Potentially cleaner water 0/-, might be unsightly, possible algae leaching	1	Environment, cleanliness, healing, justice, safety,	Flyer Newspaper Door to door Website
Environmental Groups - water quality, plant diversity, conservation	+, pushes the sustainability agenda and keeps waters clean -, concern for what to do with the contaminated biomass	1	Environment, community, responsibility, preservation	Email, check in beforehand. Send thesis if interested
Disadvantaged / Minority populations- those who do not have accesses to clean water	+, Could provide a system to clean water in their community; 0, may be indifferent if it does not change much; -, may be unsightly, possible algal leaching	1	Family, freedom, justice, unity	Same as rural areas. Possibly a presentation in schools to get students interested.

Heavy metal wastewater generating organizations	+, sustainable option, possibly cheaper 0/-, extra cost to switch treatment methods	3	Money, efficiency	Email, phone calls. Send report and data analysis
MD NRCS	0/+, similar to EPA	2.5	Environment, standardization	Contact through their website
Wastewater treatment plants downstream	0/+, system may further decreases load, WWTP may see no difference in influent pollution	2	Efficiency, money, quality over quantity	Email, phone calls. Send report and data analysis
Those responsible for wastewater treatment/ technology/ products	+, could make their job easier -, could be financial competition	3	Money, efficiency, systemization	Email Phone call Send data analysis
Health officials - national and local	+, removal of heavy metals from water will improve health of those that drink water	2.5	Healing, personal health	Email, phone calls.
Researchers who study heavy metal precipitation/ chemical wastewater treatment	0, the study connects with their end goal, but through a different method. The research could be useful to them but may not be	1	Education, discovery, collaboration	Emails, send thesis

7.2 Material List

Material	Source
5 gallon plastic bucket	Home Depot
2’ by 4’ plywood sheets	Home Depot
2x4 wood beams	Home Depot
Schedule 40 PVC (of various sizings): Pipe, Elbow joints, Couplings, Ball Valve	Home Depot
Everbilt Blue Tarp	Home Depot
DAP Ultra Clear All Purpose Waterproof Sealant	Home Depot
3”-2” PVC Schedule 40 Coupler	Lowe’s
TIMESETL 304 Stainless Steel Woven Wire 120 Mesh - 12"X40" Filter Screen Sheet Filtration Cloth	Amazon
Cheesecloth, Grade 90, 45 Sq Feet, Reusable, 100% Unbleached Cotton Fabric, Ultra Fine Cheesecloth	Sceng from Amazon
1000 ug/mL Chromium, #AACR1-125ML	Inorganic Ventures
1000 ug/mL Cadmium, #AACD1-125ML	Inorganic Ventures
Environmental Express FlipMate 100 SC0308 Filtration Assemblies, #EW-35202-33	Cole-Parmer
Qualitative Filter Paper Circles, 2.5 micron, 9cm diameter, #1005-090	Whatman from Amazon
Sand, quartz standard, washed, calcinated (Ottawa), #EM-SX0075-3	Millipore Sigma
Calcium Chloride, #10043-52-4	sciencecompany.com
Whatman GD/X 1 micron syringe filters	UMD Environmental Engineering
Arsenic solution	UMD Environmental Engineering

8 – Glossary

Absorption: process where the heavy metal is contained inside the algae

Adsorption: process where the heavy metal adhere to the surface of the algae

Biosorption: an emerging treatment method that utilizes biological agents such as bacteria and algae for the removal of contaminants, including heavy metals and nutrients

Biosorbent: the biological materials used in the process of biosorption which includes bacteria and algae

Functional Group: can be found on polysaccharide accessories, and plays a key role in the process of biosorption by binding heavy metal cations

ICP: inductively coupled plasma spectroscopy

Polysaccharide Accessories: sugar complexes located on cell walls that contain functional groups which are used in heavy metal removal

ppm: parts per million (unit of measurement equivalent to mg/L)

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